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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
The point of contact between the extracellular matrix (ECM) and specific cell surface proteins (i.e. integrins) occurs at specialized structures termed focal adhesions, which include several signal transduction molecules such as focal adhesion kinase (FAK). Intracellular signaling pathways that are responsive to ECM attachment influence cell proliferation and are altered in cancer cells. For example, FAK is overexpressed in invasive and metastatic human tumors. Furthermore, FAK is elevated in metastatic prostate cancers and preferentially associated with tyrosine-phosphorylated paxillin, a direct target of FAK. We have found that in addition to its localization to focal adhesions, paxillin is also present within nuclei and can target to the nuclear matrix of CV-1 cells, cultured prostate cancer cell lines and human prostate tissue. Furthermore, paxillin functions as a coactivator for androgen receptor and glucocorticoid receptor, but not estrogen receptor and was found to directly interact with the androgen receptor via its carboxyl-terminal LIM domain. Thus, paxillin, a protein that responds to the metastatic state of prostate cancer cells, can directly influence androgen receptor transactivation. Future studies will be directed towards the assessment of ECM effects on this novel nuclear function of paxillin as it relates to androgen action

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Introduction

Androgens are required for the growth and development of the normal prostate gland (1) but also participate in the progression of prostate cancer, particularly in early stages (2). The principal mediator of androgen action within cells is the androgen receptor (AR), a member of a large super-family of nuclear receptors (3,4). Physiological effects of androgens result primarily from transcriptional regulation of specific target genes brought about by AR interaction with specific DNA sequences, or receptor associations with other transcription factors (5-7) or coactivators (8).

The AR has proven to be a particularly useful reagent for the identification of novel transcriptional coactivators (9-12). For example, Hic-5/ARA55 was first identified as a coactivator for AR (13) and later shown to potentiate the transactivation activities of a subset of steroid hormone receptors (14) including the glucocorticoid receptor (GR). While Hic-5/ARA55 was shown to associate with the nuclear matrix (14), most studies of this protein have focused on its focal adhesion binding (15,16). Hic-5/ARA55 is a member of the paxillin family of focal adhesion proteins (17,18) that share a closely related C-terminal LIM domain region (19). In addition to their localization within focal adhesions, members of the group 3 LIM domain family are also associated with the nucleus (16,20). In spite of the prevalence of nuclear group 3 LIM domain proteins, the physiologically relevant transcriptional targets regulated by these proteins remain largely undefined.

Body

A major focus of experiments outlined in Specific Aim 1 (i.e. Task 1) was to establish whether paxillin is a coactivator for AR in prostate cancer cell lines. We have found that the transactivation activity of AR on the androgen-responsive mouse mammary tumor virus (MMTV) promoter is potentiated in transiently transfected CV-1 monkey kidney fibroblast cells (Fig. 1) and PC-3 prostate cancer cells (Fig. 2).

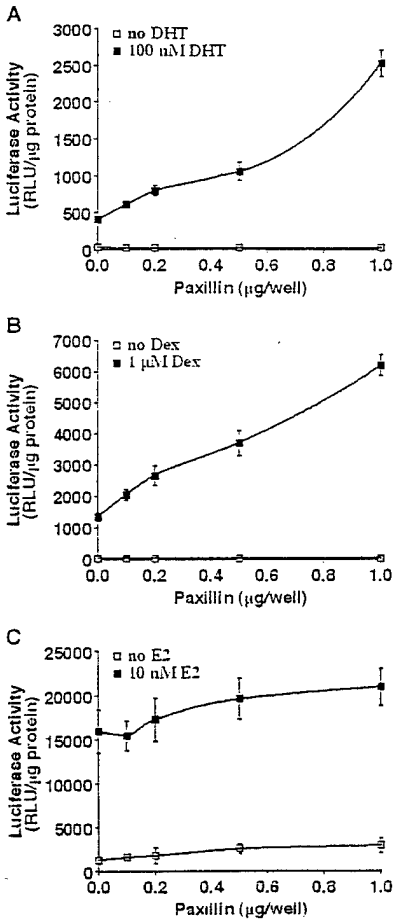

Fig. 1: Paxillin enhances AR and GR but not ER transactivation in CV-1 cells. CV-1 cells were transiently transfected with varying amounts of a paxillin expression vector along with the appropriate luciferase reporter plasmid and AR (A), GR (B) or ER (C) expression vectors. Cells incubated in the presence or absence of 10^{-7} M dihydroxyteststerone (DHT) (A), 10^{-6} M dexamethasone (Dex) (B), or 10^{-8} M 17β-estadiol (E2) (C) for 24 h. Each point and error bar shows the mean ± SD (N=4) normalized to total protein levels in each sample.

The coactivator activity of paxillin was also exerted on transiently transfected glucocorticoid receptor (GR) but not on estrogen receptor alpha (ERα) (Fig. 1). This selectivity of paxillin effects is analogous to that observed with Hic-5/ARA55, which was found to act as a coactivator for GR and AR but not ER (14).

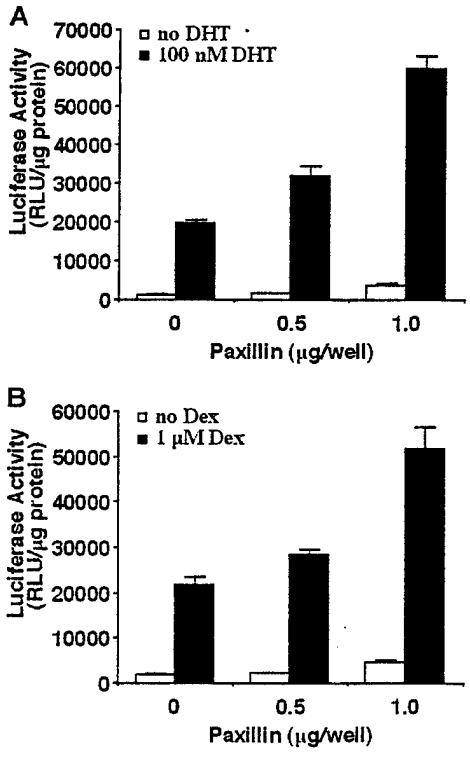

Fig. 2. Paxillin enhances AR and GR transactivation in the PC-3 prostate cancer cell line. PC-3 cells were transiently transfected with indicated amounts of a paxillin expression vector along with a promoter-linked luciferase reporter plasmid and an AR (A) expression vector or empty expression plasmid (B). A GR-expressing vector was not necessary for the GR transactivation assay since PC-3 cells express functional GR. Cells were incubated in the presence or absence of 10^{-7} M DHT (A) or 10^{-6} M Dex (B) for 24 h. Histogram shows the mean ± SD (N=4) relative luciferase activity normalized to total protein levels in each sample.

In order to identify the domain responsible for steroid receptor coactivator activity as outlined in Specific Aim1 (Task 1), paxillin amino- and carboxyl-terminal domains were transfected with AR into CV-1 cells. Results from these experiments showed that the C-terminal LIM domain of paxillin was as effective as full-length paxillin in potentiating the transactivation activity of AR and GR in CV-1 cells (Fig. 3). Basal activity of the MMTV promoter was not affected by carboxyl-terminal paxillin. In contrast to the effect of the carboxyl-terminal region of paxillin, overexpression of the amino-terminal region of paxillin did not potentiate AR transactivation in CV-1 cells (Fig. 3). However, the amino-terminal domain of paxillin was not neutral as its overexpression interfered with the coactivator activity of transfected full-length paxillin on AR and GR (Fig. 3). This "dominant negative" effect of the amino terminal region of paxillin did not result from reduced expression of transfected full-length paxillin (data not shown). Since AR and GR transactivation activity was not reduced by amino-terminal paxillin beyond that observed in the absence of overexpressed paxillin this domain of paxillin may not affect the functioning of other coactivators, at least at the levels expressed under these transfection conditions.

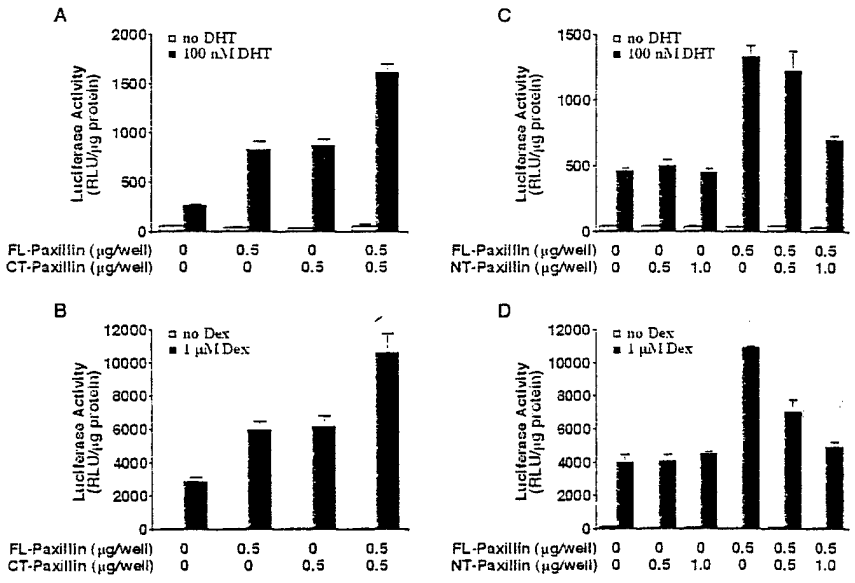

Fig. 3. Effect of paxillin C-terminus or N-terminus on AR or GR transactivation. CV-1 cells were transiently transfected with indicated amounts indicated of full-length (FL) paxillin, N-terminal (NT) paxillin, or C-terminal (CT) paxillin expression plasmids along with a promoter-linked luciferase reporter plasmid and AR (A,C) or GR expression plasmid (B,D) expression plasmids. Cells were incubated in the presence or absence of 10^{-7} M DHT (A,C) or 10^{-6} M Dex (B,D) for 24 h. Histogram shows the mean ± SD (N=4) relative luciferase activity normalized to total protein levels in each sample.

While we have not performed paxillin ablation experiments using anti-sense paxillin RNA to determine whether paxillin is an essential coactivator in prostate cancer cells, we have observed efficient AR transactivation in cultured embryonic fibroblasts derived from paxillin knock out mice (data not shown). This suggests that paxillin is not an essential coactivator for AR, at least in embryonic fibroblasts. None of the experiments involving Her-2/neu effects on AR and paxillin have been performed as of yet.

In order to assess whether paxillin effects on AR and GR are direct, we used a GST pull-down assay to examine paxillin interactions with these receptors, as outlined in Specific Aim 2 (Task 2). We found that full-length paxillin-GST fusion protein bound to AR or GR expressed in transfected CV-1 cells or the GrH2 rat hepatoma cell line, respectively (Fig. 4). Control pull-down assays with GST alone did not result in the recovery of GR or AR while the focal adhesion kinase (FAK) protein was recovered with GST-paxillin pull-downs, as expected (Fig. 4). Importantly, the *in vitro* interaction between the steroid receptors (i.e. AR and GR) and paxillin was equally efficient when receptors were unliganded or hormone bound (data not shown). Furthermore, the interaction between the steroid receptors (i.e. AR and GR) and paxillin occurred through its carboxyl-terminal LIM domain. This result is consistent with previous domain mapping of Hic-5/ARA55, which delineated its carboxyl-terminal LIM domain region as a steroid receptor-interaction domain (14). Domains on AR that interact with paxillin remain to be established. In addition, studies to address the interaction of paxillin with DNA-bound AR remain to be performed.

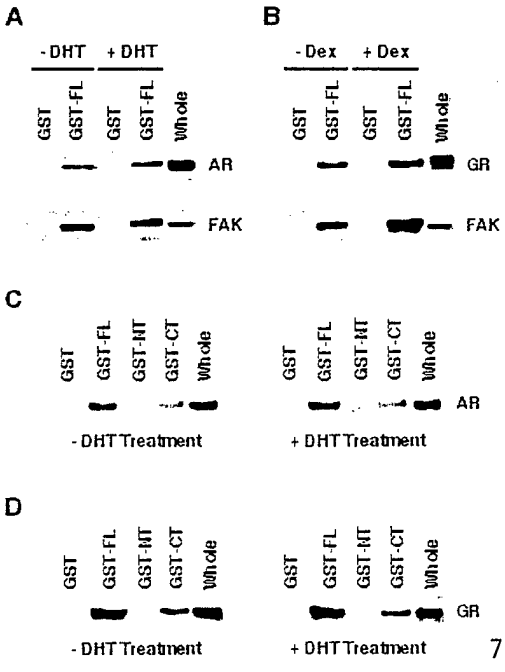

Fig. 4. Hormone-independent interaction of paxillin with steroid receptors *in vitro*. Cell lysates of CV-1 cells transfected with human AR or the GRH2 hepatoma cell line were prepared after either a 2 h hormone treatment or non-treatment and incubated with a glutathione-sepharose-bound GST-FL-paxillin (A, B, C, and D), -NT-paxillin (C and D), or -CT-paxillin (C and D) fusion protein or GST protein for 3 h. Proteins eluted from glutathione-sepharose were subjected to Western blot analysis with anti-AR antibody (A and C) or anti-GR antibody (B and D) followed by reprobing with anti-FAK antibody (A and B).

Key Research Accomplishments

Task 1:

1. Identification of paxillin as a coactivator for androgen receptor in prostate cancer cell lines
2. Identification of paxillin domain required for androgen receptor coactivation
3. Demonstration that paxillin is not an essential coactivator for androgen receptor in embryonic fibroblasts

Task 2:

4. Demonstration of a direct interaction between androgen receptor and paxillin *in vitro*
5. Identification of paxillin domain that interacts with androgen receptor

Reportable Outcomes

- **Manuscripts**

- Kasai M, Guerrero-Santoro J, Friedman R, Leman ES, Getzenberg RH, and DeFranco DB. The group 3 LIM domain protein paxillin potentiates androgen receptor transactivation in prostate cancer cell lines. Cancer Research, *submitted* (3/03).

Conclusions

In this report we establish that paxillin, the founding member of group 3 LIM domain family, can be localized within the nucleus and in particular possesses nuclear matrix binding activity. In addition, we also show that paxillin can act as a coactivator for AR in fibroblasts and prostate cancer cell lines. Thus, paxillin and Hic-5/ARA55 have the capacity to function both at

focal adhesions to aid in the cell's interpretation of signals emanating from the ECM and within the nucleus to potentiate the transactivation activity of AR. It is well established that cellular proliferation and migration is very sensitive to signals that derive from the unique ECM composition of individual tissues. Such ECM-directed signals are thought to have a critical role in the progression of cells to a metastatic state. Our results implicate particular candidate proteins (i.e. group 3 LIM domain proteins) that may serve as direct transducers of ECM signals to the nucleus. Future work as outlined in the proposal will be directed towards establishing a link between plasma membrane and nuclear functions of paxillin and Hic-5/ARA55. If indeed these proteins act as coactivators that respond selectively to ECM signals, they could serve as potential highly specific therapeutic targets that seek to ablate AR responses to the ECM and reduce the metastatic potential of prostate cancer cells.

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